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Dear Editor

Eliciting Eosinophil CCR3 Expression by Synthetic Retinoids

Over the past several decades, information about the mechanisms of eosinophilic inflammation has been accumulating. Eosinophils in tissue are supplied by blood because they are terminally differentiated, non-dividing cells. Eosinophils that accumulate at sites of inflammation contribute to tissue injury, vascular leakage, mucus secretion, and tissue remodeling by releasing their cytotoxic granule proteins, reactive oxygen species (ROS), and lipid mediators.[1] A seven transmembrane-spanning G-coupled receptor, CCR3, is selectively expressed on eosinophils and plays an important role in the trafficking of eosinophils from circulation to tissue both in healthy individuals and those with allergic reactions.

Retinoids are natural and synthetic analogues of vitamin A. In a series in our previous study, we found that naturally occurring retinoids, retinoic acids (RAs), elicit CCR3 expression on human blood eosinophils.[2] Peripheral blood eosinophils constitutively express nuclear receptors for retinoids, RA receptor (RAR)-α, -β, and -γ, and retinoid X receptor (RXR)-α and -β.[3] Using a gene microarray, we have screened up-regulation of CCR3 transcription in RA-stimulated eosinophils. Indeed, RAs have a significant effect on inducing cell surface expression of CCR3 on eosinophils. Furthermore, RA-induced CCR3 leads to enhanced functional capacities in terms of calcium signaling and chemotactic responses.[2] Surprisingly, RAs are the first recognized physiological CCR3 up-regulator in mature human eosinophils despite the recent progress in eosinophil biology.

It has been known that RAs play critical roles in mucosal immunity, especially modulating tissue-specific lymphocyte homing. Vitamin A deficiency decreases the number of T and B cells in the small bowel lamina propria.[4] Gut-associated lymphoid tissue-resident dendritic cells produce RAs that induce the gut homing receptors including CCR9 on lymphocytes. In this context, locally produced RAs might contribute to up-regulation of CCR3 on eosinophils to support the migration to gut mucosa where they normally reside.

To date, retinoids have been used for treatment of patients with skin diseases and acute promyelocytic leukemia. Many synthetic retinoids have been reported, the majority of which have a therapeutic purpose. To study whether synthetic retinoids have a similar effect on eosinophils, we obtained several compounds and tested their effect on CCR3 expression. Eosinophils were purified by negative selection using anti-CD16 immunomagnetic beads (MACS System, Miltenyi Biotec, Bergisch Gladbach, Germany) as previously described.[3] The experimental settings followed our prior study. Cells were incubated at 0.5×10^6 cells/ml in 10% fetal calf serum containing RPMI 1640 medium with indicated synthetic retinoids or vehicle. Since RAs affect eosinophil survival, the culture duration (18 hrs) was carefully chosen based on a prior study.[2] Nonpermeabilized eosinophils were stained with FITC-conjugated anti-human CCR3 monoclonal antibody or isotype-matched mouse IgG control antibody (DACO), followed by measurement of CCR3 expression using a FACScan flow cytometer. The effects of synthetic retinoids were studied at the concentration of 10^{-7} M, which is applied to the optimal concentration for 9-cisRA.[2]

Among the six synthetic retinoids we tested, Am80 (also called tamibarotene), IT-compounds (a kind gift from Dr. K. Shudo, Itsuu Laboratory, Tokyo, Japan),[5] and Ch55 up-regulated CCR3 expression (Table1). The flow cytometric histograms indicated that the whole population of eosinophils responded to increased CCR3 expression induced by synthetic retinoids (Am80: Fig.1). As shown in the Table 1, a potent effect was observed in Am80-stimulated cells. Am80 is a RAR-α and β agonist that was originally designed to ameliorate the side effect of RAs through its potent and more selective binding to RAR-α,[6] the activation of which is indispensable for CCR3 up-regulation on eosinophils. To support our previous notion that RXR partially compensates for the effect of RAs, a slight effect of pan-RXR agonist methoprene acid was observed. These results indicate that synthetic retinoids (RAR agonists) have a similar effect to natural retinoids, eliciting CCR3 expression on human eosinophils.

Therapeutic use of retinoids is potentially associated with inflammatory adverse effects such as retinoic acid syndrome and inflammatory bowel disease.[7] The accumulation of eosinophils in the gastrointestinal tract is a common feature of numerous gastrointestinal diseases, including inflammatory bowel disease.[1] Our data may be important in understanding the mechanisms of the clinical effects of synthetic retinoids and future drug discovery targeting eosinophilic inflammation. It is noteworthy that eosinophiliopoeisis appears to be under complex control of RAs under physiological conditions; RAs have pleiotropic effects on eosinophils according to the stage of cell differentiation. For instance, RAs inhibit the eosinophil differentiation in progenitor cells[8] and eosinophilic cell lines[9,10]; however, they prevent the spontaneous apoptosis of mature eosinophils.[3] Further studies are required to define the relevance of retinoid administration on eosinophil functions *in vivo*.

Table 1 The effect of synthetic retinoids on CCR3 expression on eosinophils

Retinoids (10^{-7} M)	Am80	IT-YA-01115	IT-M-01500	IT-YA-01201	Ch55	Methoprene acid
Activity	RARα/β agonist	RARα/β agonist	RARα/β agonist	RARα/β agonist	pan-RAR agonist	pan-RXR agonist
CCR3 expression (% of control ± SD)	142.1 ± 16.7**	136.8 ± 23.7**	138.9 ± 23.2**	137.5 ± 22.9**	125.1 ± 13.1*	121.6 ± 27.7

Data were assessed by the ratio of mean fluorescence intensity of the sample and the isotype-matched control, non-stimulated control normalized % values. Results were expressed as mean ± SD (n = 4-6, different donors). Differences of groups were evaluated using two-tailed Student's t-test. **$p < 0.01$, *$p < 0.05$ vs. control.

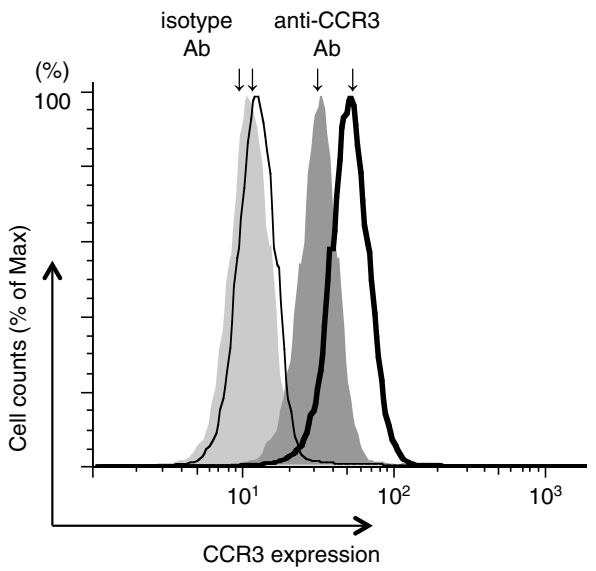

Fig. 1 The effect of Am80 on CCR3 expression on blood-derived human eosinophils. Purified eosinophils were incubated with vehicle or 10^{-7} M Am80 for 18 hrs. Representative histograms of flow cytometric analysis for surface CCR3 expression are shown (filled histograms: baseline expression in vehicle-treated control cells, open histograms: Am80-stimulated cells).

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